BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

ELSEVIER

Brain Research 828 (1999) 91–103

BRAIN RESEARCH

Research report

Differential strain susceptibility following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration acts in an autosomal dominant fashion: quantitative analysis in seven strains of Mus musculus

Kristin Hamre [a,1], Ruby Tharp [a], Kenneth Poon [a,2], Xiaoping Xiong [b], Richard J. Smeyne [a,*]

[a] Department of Developmental Neurobiology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105, USA
[b] Department of Biostatistics, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105, USA

Accepted 16 February 1999

Abstract

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used as a potent neurotoxin to approximate, in animals, the pathology that is observed in human Parkinson's disease. In this study, we examine the toxicity of MPTP in seven strains of mice, spanning a genetic continuum of Mus musculus as a prelude to uncovering complex traits associated with MPTP toxicity. Seven days following injection of 80 mg/kg MPTP (4 × 20 mg/kg every 2 h), we find that the individual mouse strains exhibit dramatic differences in SNpc neuron survival, ranging from 63% cell loss in C57BL/6J mice to 14% cell loss in Swiss–Webster (SW) mice. In order to determine if the susceptibility trait was dominant, additive or recessive, we crossed C57Bl/6J mice with either SWR/J or AKR/J mice and examined the effect of MPTP on F1 C57BL/6J × SWR/J or F1 C57BL/6J × AKR/J animals. We find that all of the F1 animals were phenotypically identical to the C57BL/6J animals. In addition, no gender differences were noted in any of the MPTP-treated inbred mice or in the F1 animals. These results suggest that susceptibility to cell loss following MPTP is autosomal dominant and this polymorphism is carried on the C57BL/6J allele.

Keywords: Parkinson's disease; Neurotoxin; Basal ganglia; Neurogenetics; Gender

1. Introduction

The syndrome, which we know today as Parkinson's disease (PD), was first described in 1817 by Dr. James Parkinson in a paper entitled 'An Essay on the Shaking Palsy' [56]. The symptoms of PD include an expressionless 'mask-like' face, rigidity of extremities or 'cogwheeling', bradykinesia, shuffling, festinating gait and lack of coordination, resting tremor (4–7/s) or 'pill-rolling', and stooped posture [14]. The pathology underlying the symptoms include degeneration of dopaminergic neurons from the substantia nigra pars compacta (SNpc) [55,71,82], presence of Lewy bodies in the regions of degeneration [20,28], and loss of inhibitory dopaminergic pathway projections from the SNpc to the caudate nucleus (in the striatum) [21]. The timecourse of the disease is progressive and fatal, although the symptomatic disease state can last for up to 20 years.

The etiology of PD is, for the most part, unknown. It is estimated that only a small percent of Parkinsonism (estimates vary from 1–10%) has a clear single gene basis [48]. Elucidation of the mutant genes, their translated proteins and signaling pathways may provide inroads to the genetic underpinnings of PD but most of all known Parkinson's disease cases are idiopathic. Current hypotheses, however, posit that PD is induced by a genetic susceptibility coupled with the interaction of an unknown agent.

One method that can provide important information regarding both genetic and environmental factors in PD is the use of animal models. Perhaps the best recapitulation of the human PD pathology in an animal model is that observed following injection of 1-methyl-4-phenyl-1,2,

* Corresponding author. Fax: +1-901-495-3143; E-mail: richard.smeyne@stjude.org

[1] Current address: Department of Anatomy and Neurobiology, University of Tennessee-Memphis School of Medicine, 855 Monroe Avenue, Memphis, TN 38163, USA.

[2] Current address: Washington University Box #3047, 6515 Wydown Blvd., St. Louis, MO 63105, USA.

0006-8993/99/$ - see front matter
PII: S0006-8993(99)01273-1

3,6-tetrahydropyridine (MPTP) [10,44]. MPTP has been demonstrated to exert its neurotoxic effects in humans and other primates, cats, and in some rodents. In rodents, it has been shown that only specific strains of mice are sensitive to the administration of MPTP [23,27,33].

In this study, we compared the differences and similarities in normal SNpc as well as the effect of MPTP on this structure in seven strains of mice spanning the evolutionary continuum of Mus musculus [81]. While a few of these mouse strains have previously been examined, no careful screen has been done in terms of evolutionary differences as a genetic tool. In this study, we addressed the following questions: (1) Is the number of SNpc neurons similar or different between the various strains of Mus musculus? (2) Are there rostral–caudal differences in the distribution of these neurons? (3) Do these seven strains of mice exhibit differential effects following administration of MPTP? (4) Are there gender differences in MPTP-induced SNpc cell loss? (5) Is the sensitivity to MPTP a dominant or recessive trait?

Our results demonstrate that different strains of mice can exhibit significantly different numbers of tyrosine hydroxylase-positive cells in the SNpc following MPTP-treatment. Moreover, MPTP-induced cell loss appears to be similar throughout the rostral–caudal extent of the SNpc. We also find that males and females within similar strains exhibit no differences in MPTP-induced cell loss. Examination of C57BL/6J × SW F1 animals following administration of MPTP suggests that sensitivity to this toxin is a dominant trait.

2. Materials and methods

2.1. Mice

All mice used in these experiments were between 3–5 months of age. Seven inbred strains of mice were examined: AKR/J, C3H/HeJ, C57L/J, C57BL6/J, DBA/1J, DBA/2J, and Swiss–Webster as well as F1 offspring from a C57BL/6J × Swiss Webster (SWR/J) cross. The F1 animals were derived both from male C57BL/6J × female SWR/J as well as female C57BL/6J × male SWR/J mice. F1 crosses from the C57Bl/6J × AKR/J were also from reciprocal matings. AKR/J, C3H/HEJ, C57L/J, C57BL6/J, DBA/1J, and DBA/2J mice were obtained from The Jackson Laboratory (Bar Harbor, ME), while Swiss-Webster mice were obtained from either the Jackson Laboratories (SWR/J) or Charles River Laboratories (Crl:CFW®SW:BR) (Wilmington, MA). C57BL/6J × SWR/J F1 and C57Bl/6J × AKR/J F1 animals were generated within our vivarium at St. Jude Children's Hospital. All animals were maintained on a 12:12 h light:dark cycle in our vivarium with ad libitum food and water and treated in accord with St. Jude Animal Care and Use Committee requirements.

2.2. MPTP treatment

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was obtained from Sigma (St. Louis, MO) and dissolved in sterile saline to a final concentration of 5 mg/ml. Each animal was given a total of 80 mg/kg of MPTP, using a dosage regimen of 4 equal subcutaneous injections of 20 mg/kg, one given every 2 h. Mice were monitored over the next 24 h for the degree of mortality and the presence of seizures. All mice were allowed to survive for one week after MPTP injection.

2.3. Histology

One week following treatment with MPTP, mice were anesthetized with an overdose of Avertin. Following induction of deep anesthesia, animals were intracardially perfused with physiologic saline followed by 4% paraformaldehyde in 1 × phosphate-buffered saline (PBS), pH 7.4. Each brain was dissected out of the skull following perfusion and post-fixed overnight in fresh fixative. Brains were then dehydrated through a graded series of ethanols, defatted in mixed xylenes and embedded in paraffin (Paraplast-X-tra™, Fisher Scientific). Brains were blocked and cut in the coronal plane and serially sectioned at 5 μm. Every section from the rostral hippocampus to the anterior aspects of the cerebellar-midbrain junction was saved and mounted onto Superfrost-Plus slides (Fisher Scientific).

2.4. Immunocytochemistry

Standard tyrosine hydroxylase (TH) immunocytochemistry was performed on all sections throughout the substantia nigra. Briefly, sections were deparaffinized through mixed xylenes and a descending series of ethanols. All antibody solutions were mixed in phosphate-buffered saline (PBS, pH 7.4) with 0.2% Triton-X100 in 0.5% bovine serum albumin (Fraction V, Sigma). Washes, except for those following the avidin-biotin complex, were also conducted in the same solution. The primary antibody was obtained from EugeneTech (NJ) and used at a dilution of 1:1000. Incubation with the primary antibody was conducted at 41° for 1 h. Standard avidin–biotin immunocytochemistry was conducted using the Vectastain ABC rabbit kit (Vector Laboratories). Immunolabeling was visualized using 3.3′ diaminobenzidine as the chromagen. All sections were counter-stained with the Nissl stains Cresyl violet or neutral red to insure that any decrease in cell numbers was not simply due to a decrease in tyrosine hydroxylase activity. All slides were subsequently dehydrated through ethanols, cleared in xylenes and coverslips applied with Permount.

2.5. Cell counting

Brains from all of the surviving animals from the MPTP injections as well as control animals were serially sec-

tioned from the rostral hippocampus to the anterior aspects of the cerebellar-midbrain junction. All of the sections were immunostained with α-TH and all of the sections from the rostral to the caudal boundaries of the SN were identified [27,35]. Each microscope slide contained five 5 μm sections which sampled 25 μm of tissue. In some cases, the TH immunostaining of MPTP-treated and control animals was suboptimal that made critical counting of the TH-positive neurons difficult. These animals were not included in the final counts presented in Table 1, but were used for the measurement of SNpc length as well as in the toxicity measurements. Of the optimally stained brains, TH-positive cells within the SNpc in one section per slide were counted using a 20× objective (total magnification 200×). Cells were counted as present within a section if they exhibited DAB reaction product in the cytoplasm and there was a clear and complete nucleus with nucleolus present. Once sections in each measured structure were counted, we summed the cell totals, multiplied by 5 to correct for uncounted sections and corrected for split nuclei using a modified Abercrombie correction factor [1,41].

To generate the nuclear size correction factor, 200 cells, sampled from the rostral-to-caudal boundaries of the SNpc, were measured by tracing the outline of the nucleus onto paper with a drawing tube and measuring the particle at its largest diameter using a 1 mm calibration slide. The 200 values were summed and a mean ± S.E.M. was generated. Comparison of cell size was made using a Student's *t*-test.

2.6. Statistical analysis

An initial comparison was made between MPTP-treated and non-treated controls in all seven strains of mice using an unbalanced ANOVA by effects model [51,67]. Further analysis focused on the Swiss–Webster, C57Bl/6J and the F1 generates from crosses of these parental strains also using an unbalanced ANOVA by effects model comparing the relationship between the number of SNpc cells and the two factors: toxin (yes/no for MPTP) and their interaction. The analysis was carried out by the general linear model (GLM) procedure because numbers of observations in each cell of mice–toxin combination were unequal. Estimations for reduction of SNpc cells in each strain of mice were generated and comparisons of the reductions of SNpc cells from MPTP-untreated to treated mice among three strains of mice using multiple comparison procedure were performed.

3. Results

Seven strains of inbred mice and two outbred (C57BL/6J × SWR/J F1 and C57Bl/6J × AKR/J F1) strains were injected subcutaneously (SC) with a total of 80 mg/kg MPTP, spread over 4 injections of 20 mg/kg every 2 h. Within one h of the first injection, all of the

Table 1
Number of SNpc cells in MPTP-treated and untreated mice

Strain	MPTP (Y/N)	n	Number of cells ± S.E.M.	% cell loss	Statistics
Swiss–Webster	N	6	3459 ± 230		1, 2, 3
Swiss–Webster	Y	4	2991 ± 236	14	
AKR/J	N	4	3623 ± 224		1, 2, 3
AKR/J	Y	3	2962 ± 89	18	
C57L/J	N	4	3415 ± 56		1, 2, 3
C57L/J	Y	4	2302 ± 138	24	
DBA/2J	N	4	3044 ± 159		1, 2, 3
DBA/2J	Y	4	2352 ± 540	23	
C3H/HeJ	N	4	3325 ± 591		4, 5, 6
C3H/HeJ	Y	4	1284 ± 171	39	
C57BL/6J	N	5	3546 ± 106		4, 5, 6
C57BL/6J	Y	7	1470 ± 230	58	
DBA/1J	N	6	3325 ± 591		4, 5, 6
DBA/1J	Y	4	1748 ± 310	53	
C57BL/6J × SWR/J F1	N	4	4237 ± 428		4, 5, 6
C57BL/6J × SWR/J F1	Y	5	1635 ± 168	61	
C57Bl/6J × AKR/J F1	Y	5	1668 ± 275	53	3, 5, 6

1: No difference between individual strain and MPTP-treated strain.
2: No significant difference between SWR/J control and other stain control.
3: No significant difference between SWR/J MPTP-treated and other strain MPTP treatment.
4: Individual control strain significantly different ($p \leq 0.05$) from MPTP-treated strain.
5: No signficant difference between MPTP-treated C57Bl/6J and individual strain.
6: Significantly different ($p \leq 0.05$) from SWR/J MPTP-treated mice.
Total number of tyrosine hydroxylase-positive substantia nigra pars compacta cells of seven strains of mice as well as 1 outbred C57BL/6J × SWR/J F1 strain prior to and following 80 mg/kg SC MPTP.
The percent reduction is the ratio of each individual strain following MPTP divided by the number of TH-positive SNpc neurons in non-treated animals. Statistical comparison was done using an unbalanced ANOVA by effects model followed by a 2-tailed Student's *t*-test for individual comparisons.

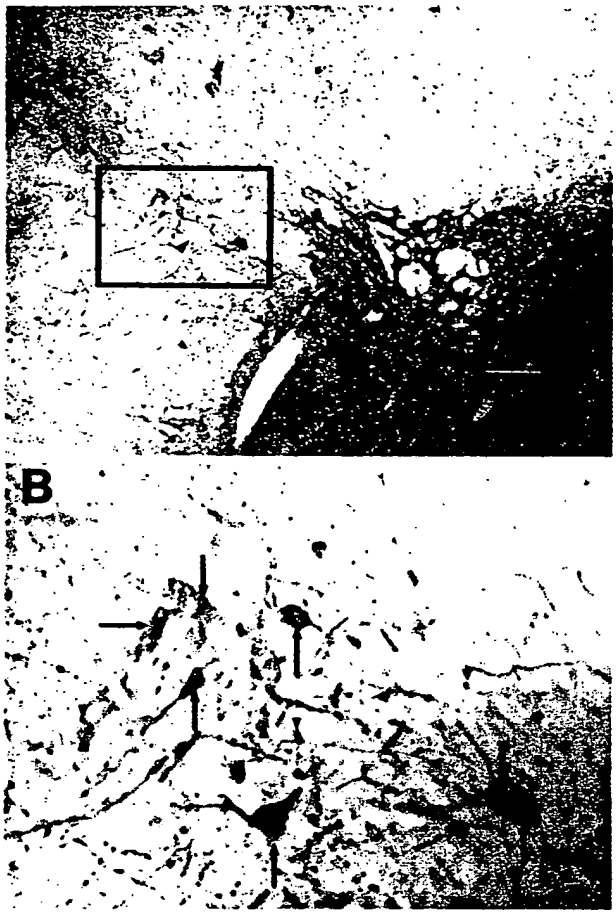
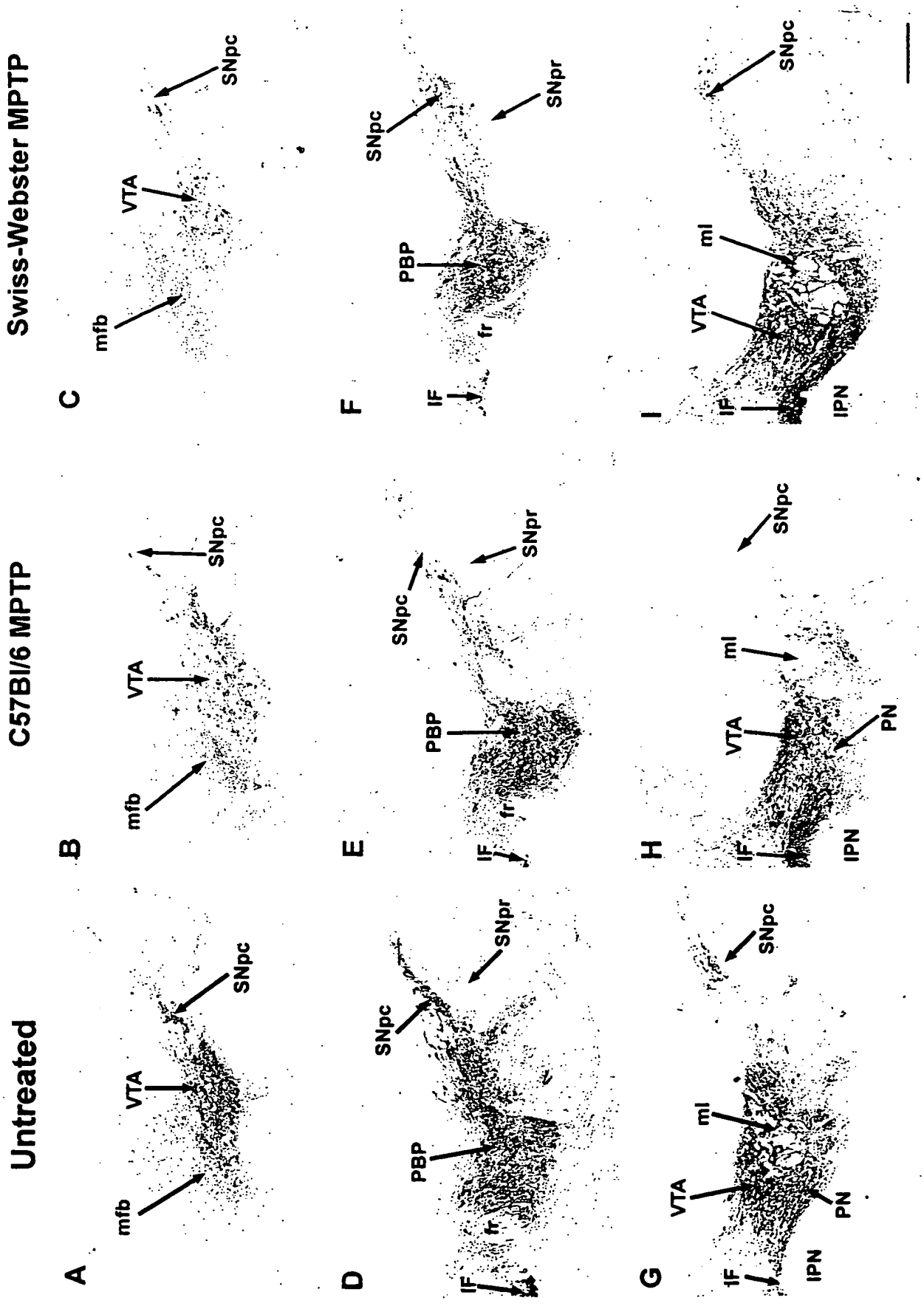


Fig. 1. TH-immunohistochemistry followed by Neutral Red Nissl staining demonstrating cell loss in the C57BL/6J MPTP-treated mouse. A) In this section, there is a severe depletion of TH-positive staining in the SNpc box). B) High power magnification of the box shown in A). Large TH-positive SNpc neurons arrows) are visualized by brown DAB reaction product in their cytoplasm surrounding a relatively unstained nucleus. Small. Nissl-stained non-dopaminergic cells. consisting mainly of presumptive glia arrowheads) are also present. No TH-negative cells with the size or shape characteristics of SNpc cells were present. Scale bars A) = 150 m. B) = 35 m.

animals from each of the strains appeared somewhat lethargic. After the second injection, we observed that animals of some strains died of what appeared to be respiratory/cardiac arrest. This peripheral toxicity was greatest in the C57BL/6J strains 6/15 animals died. resulting in a 40% toxicity). followed by DBA/1J 1/4. 25%). C3H/HeJ 1/9. 11%) and Swiss-Webster combined SWR/J and Crl:CFW SW:BR. 1/9. 11%) and AKR/J 0/8. 0%). Quite a few DBA/2J animals died during the experiment 7/11. 64%); however. the mode of death appeared to be due to a limbic-type status epilepticus rather than respiratory arrest. Although beyond the scope of this manuscript, it has been shown that this strain is sensitive to audiogenic seizures [12] as well as to limbic-seizures induced by stimulation of nicotinic acetylcholine receptors [47]. The peripheral toxicity of the SW × C57-BL/6J F1 cross was 38% 3/8) and the C57Bl/6J × AKR/J was 29% 2/7).

Seven days following SC injection of MPTP, animals were sacrificed and the brains of each animal was processed for tyrosine hydroxylase TH) immunohistochemistry in order to quantify the number of cells present in the SNpc. Every 5 m paraffin section through the SNpc [35,52] was immunostained for TH. In addition to staining with anti-TH, all sections were counterstained with either neutral red or Cresyl violet Nissl stains) so that any non-TH cells that appeared to be neuronal-like could also be counted. However, we did not observe any TH-negative, Nissl-positive cells with similar size and/or characteristics as those seen in normal SNpc neurons Fig. 1), similar to the observation of German et al. [27] in any of the mouse strains that were quantified. The average particle size for the TH-positive SNpc neuron, which in this study was the unstained nucleus, was 12.8 m in all of the non-treated strains of mice as well as in the non-sensitive strains of MPTP-treated mice Swiss–Webster, AKR/J, C57L/J). The average particle size was 11.8 m in MPTP-treated mice from sensitive strains DBA/2J, C3H/HeJ, C57BL/6J, DBA/1J and C57BL/6J × SW F1). While the basis of the nuclear shrinkage in the sensitive strains of mice is at this time unknown, the change in nuclear size is statistically significant two-tailed Student's t-test, $p \leq 0.001$, $t = 7.586$, df = 199). Using the modified Abercrombie cell correction factor for split nuclei [1,41], we calculated correction factors for raw number counts to be 0.281 for all non-treated and non-sen-

Fig. 2. Normal distribution of tyrosine hydroxylase-positive cells in the midbrain of the untreated A, D, G) and MPTP-treated C57BL/6J B, E, H) and Swiss-Webster C, F, I) mouse through the rostral A, B, C), intermediate D, E, F) and caudal G, H, I) substantia nigra pars compacta SNpc). A) The rostral limits of the SNpc lie lateral to the medial forebrain bundle mfb) and ventral tegmental area VTA). B) Section through the rostral SNpc at the level shown in A) following MPTP treatment in a C57BL/6J animal. A large reduction in cells is seen in SNpc, with little effect on the adjacent VTA. C) In the Swiss–Webster animal, the rostral structure of the SNpc is virtually identical to the untreated mouse A) D) Approximately midway through the substantia nigra, the cells of the pars compacta are situated immediately superior to the substantia nigra pars reticularis SNpr), lateral and rostral to the nucleus parabrachialis pigmentosus PBP), fasciculus retroflexus of Meynart fr) and interfascicular nucleus IF). E) At the level shown in D), the SNpc from a C57BL/6J MPTP treated mouse shows a large depletion of TH-positive cells. Little change is seen in the surrounding dopaminergic structures. F) In the Swiss–Webster animal, the intermediate structure of the SNpc is virtually identical to the untreated mouse D) G) Section through the caudal substantia nigra. At the level of the nucleus paranigralis PN), medial lemniscus ml) and interpeduncular nucleus IPN), the SNpc is situated in the lateral midbrain. H) At the caudal limits of the SNpc, there is a virtual depletion of substantia nigra neurons following MPTP treatment. No qualitative changes were detected in the adjacent dopaminergic VTA or PN. I) In the Swiss–Webster animal, the caudal structure of the SNpc is virtually identical to the untreated mouse G). Scale bar A–I = 275 m.


Untreated
C57Bl/6 MPTP
Swiss-Webster MPTP
A
B
C
D
E
F
G
H
I
SNpc
SNpr
VTA
mfb
PBP
fr
IF
ml
PN
IPN

sitive strains of mice and 0.291 for the MPTP sensitive strains of mice.

3.1. Quantification of normal and MPTP-treated substantia nigra pars compacta

In non-treated animals, all strains of mice had similar numbers of TH-immunopositive SNpc neurons. Seven days following i.p. injection of MPTP, the greatest percentage of cell loss was seen in the C57BL/6J mice, followed in an almost linear continuum by DBA/1J, C3H/HeJ, and DBA/2J mice. Although some level of cell loss was detected in all of the other strains of mice examined (see Table 1), MPTP had no effect on SNpc neuron numbers in Swiss Webster, AKR/J or C57L/J strains of mice. Photographic examples of TH-positive cell loss in the SNpc from a sensitive strain (C57BL/6J) and an insensitive strain (Swiss–Webster) are shown in Fig. 2.

In order to determine if the effects of strain were dominant, recessive or additive, we examined the effect of SC injection of MPTP on animals generated by an F1 cross between our most sensitive (C57BL/6J) and resistant strains (Swiss–Webster) of mice. These two strains of mice were chosen because they start with statistically identical numbers of SNpc neurons, but dichotomize in their effect to MPTP. The SNpc from C57BL/6J × SW F1 animals contained approximately the same number of TH-positive neurons as were present in both the Swiss-Webster or C57BL/6J animals. However, 7 days following SC injection of MPTP, each of the C57BL/6J × SWR/J F1 mice demonstrated considerable SNpc cell paucity. In fact, the number of surviving neurons was similar to that of MPTP-treated C57BL/6J animals. Therefore, the addition of one allele of resistant genetic material from the Swiss Webster mouse had no attenuating effect on MPTP toxicity (Table 1). A similar result was observed using F1 crosses between AKR and C57Bl/6J animals (Table 1).

3.2. Statistical analysis of cell numbers in MPTP-sensitive and animals

3.2.1. Cell number

In the first analysis of all seven strains of mice, analysis of variance (ANOVA) demonstrated that the number of SNpc cells was significantly affected by strain ($P = 0.004$), toxin ($P = 0.0001$), and their interaction ($P = 0.0024$). In other words, the mean number of SNpc cells following MPTP was significantly different among the strains of mice, and effect of toxin was significantly different between the various strains of mice. For untreated mice, the mean numbers of SNpc cells in all strains of mice was not significantly different ($P = 0.09$) at level alpha = 0.1 (See Table 1).

The goal of this work was to identify both the most- and least-sensitive strains of Mus musculus following administration of MPTP. From this analysis we determined that the Swiss–Webster was the least sensitive and C57Bl/6J was the most sensitive. Therefore, all further statistical analysis was performed using these strains of mice as well as their F1 progeny.

The mean reduction of SNpc neurons in each strain of mice is shown in Table 1. The reduction of SNpc cells was not significant for Swiss–Webster ($P = 0.2$), but was significant for C57Bl/6J ($P = 0.0001$) and for C57Bl/6JxSWR/J F1 and C57Bl/6J × AKR/J ($P = 0.0001$). By the contrast test, the reductions of SNpc cells were different among the three strain of mice ($P = 0.0024$).

3.3. Normal distribution of SNpc neurons in different strains of Mus musculus

3.3.1. Untreated animals

The rostral-to-caudal length of the SNpc material in our formaldehyde-fixed paraffin embedded in the seven strains of mice examined ranged from 700–850 μm. The rostral-to-caudal length of the SNpc appeared to have no impact on cell number or sensitivity to MPTP as Swiss-Webster and C57BL/6J both have the smallest length (700 μm), followed by C57L/J (750 μm), then DBA/2J, C3H/HeJ and AKR/J (850 μm).

As stated above, the total number SNpc cells was similar in all of the strains (except DBA/1J) examined. However, they appeared to have different patterns of distribution (Fig. 3). For the most part, the majority of the neurons were situated in the rostral part of the SNpc, encompassing an area from the level of the medial forebrain bundle through the nucleus parabrachialis pigmentosis. In the AKR/J, DBA/2J, and C57BL/6J strains of mice there was a progressive decline in cell number as the SNpc moves caudally through the appearance of the medial lemniscus and nucleus paranigralis to the area where the SNpc disappears at the level of the retrorubral field. This occurred, albeit to a lesser degree, in the C3H/HeJ and C57L/J strains. The pattern of cell placement in the Swiss–Webster mice was different from the other strains of mice examined in that there appeared to be a biphasic distribution of SNpc cells, such that there appeared to be an equal number of cells in the rostral part of the structure as there were in the caudal part (see Fig. 3).

3.3.2. MPTP-treated animals

In order to determine if the immediate cellular environment had a role in MPTP toxicity, we examined the effects of this neurotoxin on SNpc neurons at different rostral–caudal levels in each of the seven mouse strains. Although neurons of the SNpc are differently distributed in each of the strains, there appears to be no preferential effects of MPTP that are dependent on cell position (Fig. 3). Thus, in sensitive strains of mice, rostrally situated cells are just as sensitive as their caudal counterparts. Similarly, in the resistant strains, the position of the individual cells plays no apparent role in MPTP-toxicity.

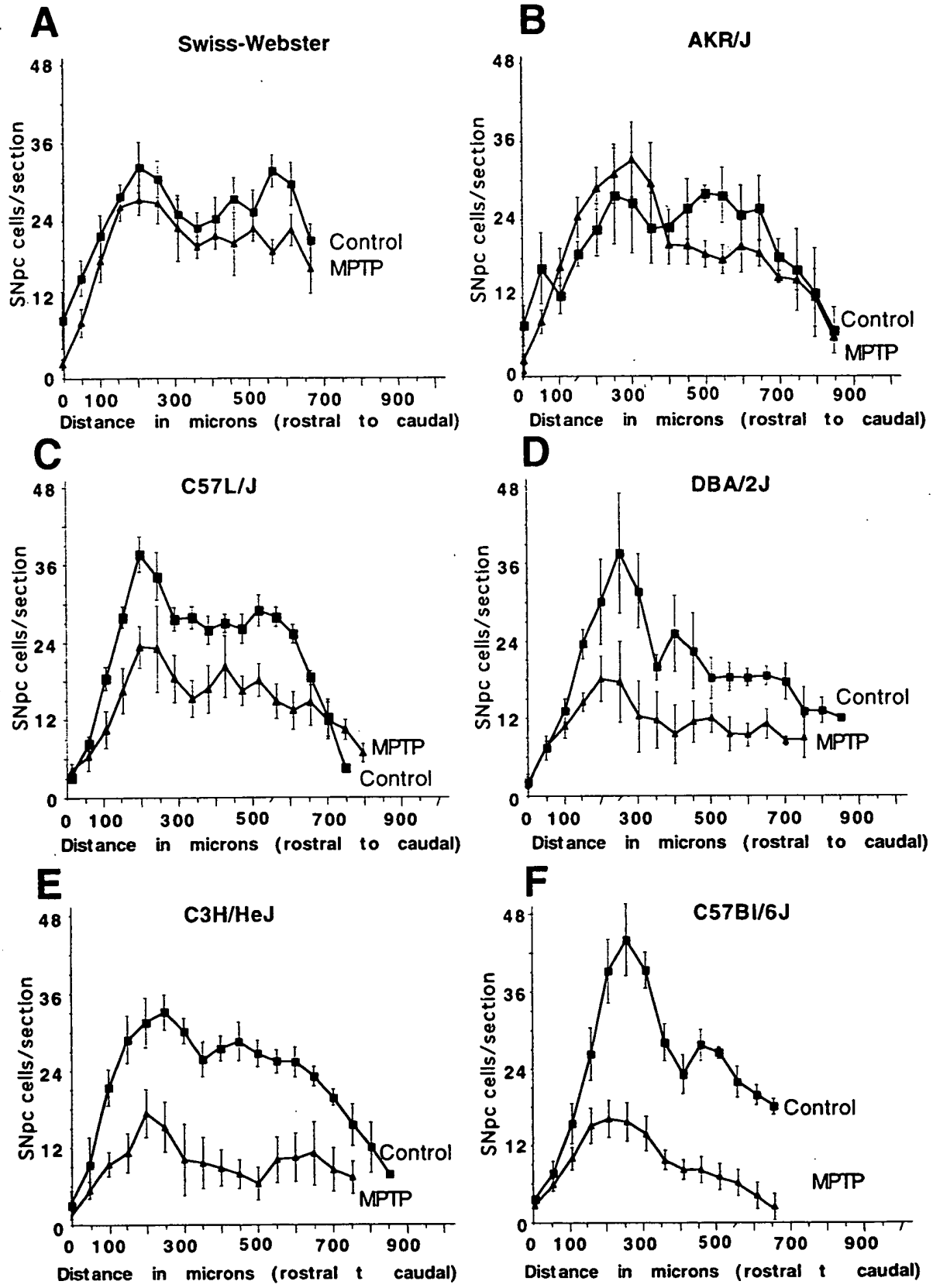


Fig. 3. Rostral-to-caudal distributions of SNpc neurons in six strains of non-treated- and MPTP-treated mice. Strains are listed (top to bottom) from least to most MPTP-sensitive. In the (A) Swiss–Webster, (B) AKR/J and (E) C3H/HeJ strains of mice, neurons are evenly distributed in the rostral and caudal halves of the SNpc. In the (D) DBA/2J, (C) C57L/J and (F) C57BL/6J strains, the majority of cells within the SNpc are situated in the rostral half of the structure.

In order to determine if the effects of strain on cell distribution in the SNpc were dominant, recessive or additive, we examined the animals generated by an F1 cross between a rostrally distributed (C57BL/6J) and evenly distributed strains (SWR/J) of mice. Examination of these F1 animals revealed that the TH-positive cells were evenly distributed in the rostral-to-caudal extent of the substantia nigra. Thus, unlike the cell loss seen in the SNpc following administration of MPTP, the distribution of substantia nigra pars compacta neurons more closely resembled that of the Swiss-Webster than the C57BL/6J animals. We also observed that the rostral-to-caudal length of the C57BL/6J × SW F1 animals was approximately 150 μm longer than either the SW or C57BL/6J animals alone (Fig. 4). This suggests that the genetic component underlying placement of cells in the SNpc involves a more complex genetic mechanism that simple Mendelian inheritability.

3.4. Role of gender in MPTP toxicity

In humans, the appearance of Parkinson's disease has a clear sexual dichotomy such that males are generally thought to have a 1.5–2.0 × greater prevalence rate than females in acquiring the disease [34,50]. In order to determine if MPTP had any gender-dependent specificity, we examined neurons loss in age-matched males and females from C57BL/6J mice; the strain that was most sensitive to the effects of MPTP. Examination of the cell loss in the SNpc 7 days following 80 mg/kg i.p. injection of MPTP, we found no differences between male and female mice (Fig. 5). We conclude that gender plays no role in MPTP toxicity. Examination of gender differences in the other six strains examined showed similar results (data not shown).

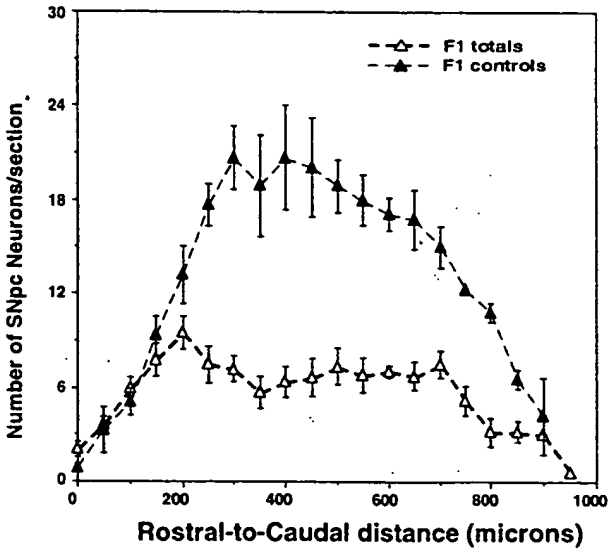


Fig. 4. Rostral-to-caudal distribution of SNpc neurons in the C57BL/6J x SW F1 untreated- and MPTP-treated animals. Unlike the cell loss seen in the F1 animals (Table 1), the distribution of cells in the F1 animals is different than both the C57BL/6J or Swiss–Webster founders. Following MPTP-treatment (open triangles), there is an approximate loss of 70% of the TH-positive neurons.

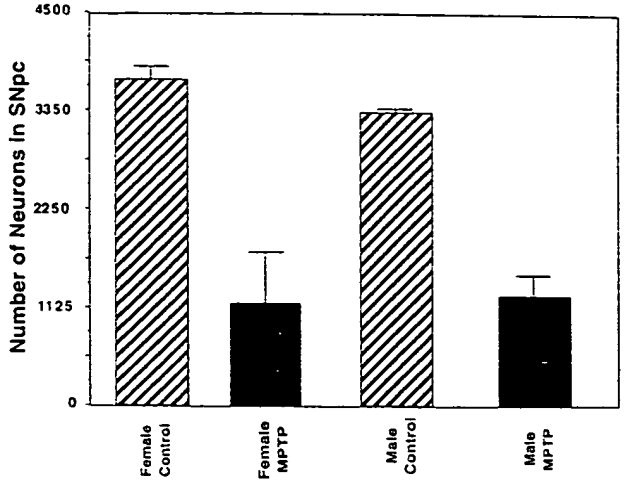


Fig. 5. Role of gender in MPTP-induced cell loss in C57BL/6J mice. The total number of SNpc neurons present in male or female untreated mice is statistically identical. Seven days following administration of MPTP, there is a loss of approximately 70% of the TH-positive neurons in both male and female animals. This finding suggests that there are no gender differences following MPTP in the most susceptible strain of Mus musculus (C57BL/6J).

4. Discussion

We find that sensitivity to MPTP, resulting in cell loss in the substantia nigra pars compacta, is dependent on the particular strain of Mus musculus to which it is administered. The most sensitive strain of mouse is C57BL/6J, while the least sensitive strain(s) are Swiss–Webster (either SWR/J or Crl:CFW®SW:BR) and/or AKR/J. Although a previous study reported that Swiss-Webster from different suppliers had variable responses to MPTP [33], we did not find any difference in response to MPTP in either of the inbred Swiss–Webster mice that we used. While it may appear that intermediate cell loss was seen in the C57L/J, DBA/2J, C3H/HeJ and DBA/1J strains of Mus musculus, this appearance is deceiving. Statistically, the data is binned into two compartments: (1) those that are not significantly different than Swiss-Webster but are significantly from C57Bl/6J and (2) those that are significantly different from Swiss–Webster but are not different than C57Bl/6J (see Table 1).

In addition, we have found that neurons within the substantia nigra pars compacta are differentially dispersed, again dependent upon strain. In C57BL/6J mice, a majority of the TH-positive neurons are rostrally placed, while in all other strains of mice examined, the TH-positive neurons are more evenly distributed. While no statistical analysis was done here, the qualitative appearance of the neuronal distribution is striking (Fig. 3). The importance of this observation in relation to MPTP toxicity is unknown

since there appears to be equal sensitivity to MPTP in rostral- vs. caudal-situated cells.

Examination of F1 crosses between C57BL/6J and Swiss–Webster mice suggest that MPTP-sensitivity is a dominant trait since all of the F1 animals examined were phenotypically identical to the C57BL/6J alone in response to MPTP. Similarly, neuron placement also appear to be a dominant trait, although the dominant allele resides within the DNA of the Swiss–Webster animal. Since the F1 animals were generated using both male and female animals of each strain, we have eliminated the possibility that any of the observed effects were due to maternal imprinting. In addition, it is unlikely that any of the contributing genes lie on the sex chromosome, and therefore would be autosomal.

In the MPTP-sensitive strains of mice, particularly the C57BL/6J animals, we found no gender differences in MPTP-induced neuronal loss. Previous studies have found gender differences; however, these studies examined dopamine levels in the striatum and not cell loss in the SNpc [24]. Given these results, it is possible that the increase in PD in males vs. females could be the result of differences in the compensatory mechanisms for striatal dopamine levels rather than differences in cell loss in the substantia nigra. Another possible explanation for the decrease in PD in females is the observation that estrogen can be neuroprotective against dopamine loss in the striatum following MPTP [19].

The differential action of MPTP within different species [38,45,46,70] and strains [23,74] has been a well studied phenomenon. The results on SNpc cell loss in the C57BL/6J mice following MPTP injection presented in this paper agrees with that presented in a previous study [27]. We also show that there is no significant cell loss in the Swiss–Webster strain, supporting previous studies that showed relatively little change in striatal dopamine levels following MPTP [77]. Additionally, we have extended the quantitative analysis of SNpc cell loss to include 5 strains of mice that previously have not been reported.

The basis for the difference in MPTP susceptibility between mouse strains is not understood. Although several explanations have been offered, none seem to fully explain this phenomenon. For example, it has been proposed that differences in monoamine oxidase (specifically MAO-B) levels could account for the differences in MPTP action since these enzymes catalyze, in glia [11], the production of the toxic MPP+ from MPTP [17,30]. Examination of MAO levels in the brains of various strains of mice show that although differences are present, these should not account for the dramatic levels of MPTP sensitivity [65,76]. Further support for the argument that the differential sensitivity to MPTP is not manifested by changes in monoamine levels is that MAO-A- or MAO-B-overexpressing transgenic mice demonstrate no alterations in their MPTP toxicity profiles compared with control founder strains [5,29]. In addition, no changes in the vesicular monoamine transporter was observed in sensitive vs. insensitive strains of mice [39].

Another possibility for the strain variability to MPTP is that the different strains have different tolerances to oxidative changes. It has been shown that MPTP increases free radical production following the inhibition of complex I in mitochondria [4,84]. This increase was age dependent and correlates with the age-related susceptibility to MPTP-induced cell loss [3,4]. Further support for the role of free radicals in MPTP-induced substantia nigra cell death is found in studies that have demonstrated that increased levels of superoxide dismutase, which protects against free radical damage, can safeguard these neurons [62]. Further support for the role of free radical formation in MPTP-induced cell death is found in studies showing that the presence of iron may contribute to the toxicity of MPTP [16] and that there are increased levels of iron in the brains of Parkinsonian humans [73,83]. In spite of the role of free radicals in MPTP-induced cell death, little is known about the different oxidation states of various mouse strains, specifically as it relates to the SNpc. One study has examined strain differences in free-radical formation through the administration of an MPTP analogue, 2′-NH2-MPTP, which affects serotinergic and noradrenergic populations of cells. Here, no differences in free-radical production were found in the striatum of C57Bl/6 and Swiss–Webster mice [6–8], suggesting that free-radical production, alone, could not explain the differences in strain susceptibility. Another study, however, used clonally related PC12 cells engineered to express dopamine and found higher levels of both free radicals and free radical damage in cells expressing higher levels of MAO-B. In addition, increased MAO-B levels within PC12 cells caused a dose-dependent increase in sensitivity to the toxin MPTP [79]. Further studies on strain variability in free radical production and tolerance are needed to resolve these conflicting studies.

In addition to free radical formation, several other potential mechanisms for differential MPTP sensitivity are being explored, including differential uptake of MPP+ through the dopamine transporter [25,58], changes in glutamate transporter function in astrocytes [32], control of calcium flux into substantia nigra cells [42], as well as functional changes in electron transport chain proteins [78]. At this time, none of these studies are conclusive as to their individual role in MPTP toxicity.

Another consideration of differential toxicity following MPTP administration is the death of animals prior to the 7-day period of neuronal cell death. Generally, we found that animals die within 8–24 h after the first SC MPTP injection. It was also noted that the animals most susceptible to death mirrored the neuronal sensitivity to MPTP. This correlation argues against a bias on cell death based on only analyzing animals that survive the procedure. If the peripheral toxicity is correlated to neuronal cell death, one could argue that the animals that died would have

been more sensitive to the action of MPTP and therefore may have had a larger cell loss. Thus, we feel that the comparisons of substantia nigra cell loss in only the living animals is still valid and in fact may be underestimating the differences between strains. As stated earlier, the cause of death in these animals is unknown but appears to be due to respiratory/cardiac distress. While differences in MAO levels are not significantly different in the brains of different mouse strains [65,76], differences in MAO-A or MAO-B levels in peripheral tissue of mice have not been explored. However, MAO levels are extremely high in heart and lung and liver [37,49,68]. In addition, in isolated hepatocytes, MPTP is rapidly converted to MPP + and is highly toxic to hepatocytes, most likely due to ATP depletion [15,72]. It is possible that a similar depletion of ATP occurs in lung and liver, which could lead to infarction and death [36,64].

Whatever the mechanism of action, our finding that the crossing of C57Bl/6J mice to 2 different resistant strains of mice (to generate heterozygous F1 animals at all alleles: F1 C57BL/6J × SWR/J and F1 C57BL/6J × AKR/J) resulted in MPTP-sensitive mice suggests that the gene sequences that underlie the MPTP susceptibility are contained within the C57Bl/6J genome and are autosomal dominant. If the trait were recessive, one would have expected all of the animals to have the resistant characteristics of the SWR/J or AKR/J animals. If there was a semidominance, then one would have expected to find a phenotype intermediate to the C57Bl/6J and the SWR/J or AKR/J animals.

Unlike the dominance of the C57BL/6J animal in regard to MPTP sensitivity, the positioning of TH-positive cells in the substantia nigra appears to be a dominant trait carried by the SWR/J animals. Here we find that in untreated and MPTP-treated F1 animals, there is a relatively uniform distribution of cells in the pars compacta. If the gene(s) that conferred cell position were C57BL/6J dominant than one would have expected to see the majority of the cells situated in the rostral part of the midbrain.

The identification of the genetic factor(s) that predispose an individual to idiopathic Parkinson's disease has been quite elusive. In fact, controversy still exists as to how much of the disease results from a strict genetic causation, a purely environmental factor, or the more parsimonious combination of the two risk factors [31].

A small number of familial parkinsonian patients appear to have an alanine-to-threonine polymorphism at position 53 in the gene encoding the α-synuclein protein [60], suggesting that this aggregating protein [75] may play a role in Lewy body formation that ultimately results in substantia nigra cell death [54]. However, at this time, no mutations in this protein have been reported in idiopathic PD. Two additional loci have been found which are linked to human PD, one located at human chromosome 2p13 [26] and another at 6, which has been shown to encode a gene named *parkin* [40]. The PD linked to this locus more closely resembles that of idiopathic PD, although like the α-synuclein protein, this unknown protein has very low penetrance.

Interest in the role of environmental factors range from the largely discredited role of viruses in the etiology of PD [61,63] to the role of neurological toxins in the environment, mostly based on the effects of MPTP [10,22,46]. MPTP acts as a poison to complex I of the mitochondrially based electron transport chain [78]. Since alterations in Complex I activity have been seen in PD [69], the role of mitochondrial genes, such as CYP2D6 which encodes the debrisoquine 4-hydroxylase cytochrome *P*450 [2,66], as well as drug metabolizing enzymes such as cytochrome P450IAI [13,43] have been examined. Although small functional changes in these individual genes are in some cases linked to higher risk of developing PD, the results are still controversial [18]. It is more likely that small polymorphic changes in multiple genes (complex traits) may be additive in PD causation.

Two methods have recently been used to identify differences between two populations in an unbiased manner. The first, differential display, has been used to compare untreated PC12 cells to those exposed to MPP + . In this preliminary study, 98 genes were differentially-expressed; 37 from the mitochondrial genome including ND-2 from Complex I, 15 from the heat shock protein family, 5 from nuclear ribosomal genes and 41 were unidentified [53]. A second unbiased method for identifying genes that contribute to any particular phenotype is the detection of quantitative trait loci. In this technique, random markers equally spaced throughout the entire genome are screened in order to find polymorphic loci that occur between two defined strains that statistically correlate with any identified quantitative measurement [57]. While early use of this technique in mammals has focused on behavioral paradigms [9,59], recent work has focused on phenotypes such as brain weight and retinal cell number [80,81]. In this paper, we performed quantitative measurements on 7 strains of Mus musculus, both in the untreated and MPTP-treated conditions, in order to determine their viability as partner strains for a quantitative trait loci analysis. C57BL/6J mice and Swiss-Webster are sufficiently different to allow this polymorphism mapping technique to be utilized. If the molecular processes that lead to SNpc cell death following administration of MPTP intersect with those that occur in idiopathic Parkinsonism, then these methods may provide an entree into uncovering the complex genetic traits that predispose an individual to this disease.

Acknowledgements

The authors thank Angeles Fernandez-Gonzales and Emmitt Major for initial help using MPTP and data reduction. We also thank Justine Cunningham and Ciaran Faherty for critical reading of this manuscript. The authors

are supported in part by NIH Cancer Center Support CORE Grant P30 CA21765 and by the American Lebanese Syrian Associated Charities (ALSAC).

References

[1] M. Abercrombie. Estimation of nuclear population from microtome sections. Anat. Rec. 94 (1946) 239–247.

[2] S.N. Akhmedova. E.A. Pushnova. A.F. Yakimovsky. V.V. Avtonomov. E.I. Schwartz. Frequency of a specific cytochrome *P*4502D6B (CYP2D6B) mutant allele in clinically differentiated groups of patients with Parkinson disease. Biochem. Moll. Med. 54 (1995) 88–90.

[3] S.F. Ali. S.N. David. G.D. Newport. Age-related susceptibility to MPTP-induced neurotoxicity in mice. Neurotoxicology 14 (1993) 29–34.

[4] S.F. Ali. S.N. David. G.D. Newport. J.L. Cadet. W. Slikker Jr.. MPTP-induced oxidative stress and neurotoxicity are age-dependent: evidence from measures of reactive oxygen species and striatal dopamine levels. Synapse 18 (1994) 27–34.

[5] J.K. Andersen. D.M. Frim. O. Isacson. M.F. Beal. X.O. Breakefield. Elevation of neuronal MAO-B activity in a transgenic mouse model does not increase sensitivity to the neurotoxin 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP). Brain Res. 656 (1994) 108–114.

[6] A.M. Andrews. B. Ladenheim. C.J. Epstein. J.L. Cadet. D.L. Murphy. Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2′-NH2-MPTP on serotonergic and noradrenergic nerve terminals. Mol. Pharmacol. 50 (1996) 1511–1519.

[7] A.M. Andrews. D.L. Murphy. 2′-NH2-MPTP in Swiss Webster mice: evidence for long-term (6-month) depletions in cortical and hippocampal serotonin and norepinephrine. differential protection by selective uptake inhibitors or clorgyline and functional changes in central serotonin neurotransmission. J. Pharmacol. Exp. Ther. 267 (1993) 1432–1439.

[8] A.M. Andrews. D.L. Murphy. Sustained depletion of cortical and hippocampal serotonin and norepinephrine but not striatal dopamine by 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH2-MPTP): a comparative study with 2′-CH3-MPTP and MPTP. J. Neurochem. 60 (1993) 1167–1170.

[9] K.J. Buck. P. Metten. J.K. Belknap. J.C. Crabbe. Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. J. Neurosci. 17 (1997) 3946–3955.

[10] R.S. Burns. P. LeWitt. M.H. Ebert. H. Pakkenberg. I.J. Kopin. The clinical syndrome of striatal dopamine deficiency; parkinsonism induced by MPTP. New Engl. J. Med. 312 (1985) 1418–1421.

[11] F.W. Chang. S.D. Wang. K.T. Lu. E.H. Lee. Differential interactive effects of gliotoxin and MPTP in the substantia nigra and the locus coeruleus in BALB/c mice. Brain Res. Bull. 31 (1993) 253–266.

[12] S.H. Chung. M.S. Johnson. Studies on sound-induced epilepsy in mice. Proc. R. Soc. London B 221 (1984) 145–168. Review: 94 refs.

[13] A.K. Daly. S. Cholerton. M. Armstrong. J.R. Idle. Genotyping for polymorphisms in xenobiotic metabolism as a predictor of disease susceptibility. Environ. Health. Persp. 102 (1994) 55–61.

[14] P.J. Delwaide. M. Gonce. Pathophysiology of Parkinson's signs. in: J. Jankovic. E. Tolosa (Eds.). Parkinson's Disease and Movement Disorders. Williams and Wilkins. Baltimore. 1993. pp. 77–92.

[15] D. Di Monte. S.A. Jewell. G. Ekstrom. M.S. Sandy. M.T. Smith. 1-Methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridine (MPP+) cause rapid ATP depletion in isolated hepatocytes. Biochem. Biophys. Res. Commun. 137 (1986) 310–315.

[16] D.A. Di Monte. H.M. Schipper. S. Hetts. J.W. Langston. Iron-mediated bioactivation of 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP) in glial cultures. Glia 15 (1995) 203–206.

[17] D.A. Di Monte. H.M. Schipper. S. Hetts. J.W. Langston. Iron-mediated bioactivation of 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP) in glial cultures. Glia 15 (1995) 203–206.

[18] N. Diederich. C. Hilger. C.G. Goetz. M. Keipes. F. Hentges. e.P. Vieregg. H. Metz. Genetic variability of the CYP 2D6 gene is not a risk factor for sporadic Parkinson's disease. Ann. Neurol. 40 (1996) 463–465.

[19] D.E. Dluzen. J.L. McDermott. B. Liu. Estrogen as a neuroprotectant against MPTP-induced neurotoxicity in C57/B1 mice. Neurotoxicol. Teratol. 18 (1996) 603–606.

[20] M.J. Eadie. The neuropathology of Parkinson's disease. Aust. New Zealand J. Med. 1 (1971) 7–13. Suppl. 1.

[21] J.M. Fearnley. A.J. Lees. Striatonigral degeneration. A clinicopathological study. Brain 113 (1990) 1182–1842.

[22] L.S. Forno. L.E. DeLanney. I. Irwin. J.W. Langston. Similarities and differences between MPTP-induced parkinsonism and Parkinson's disease. Neuropathologic considerations. Adv. Neurol. 60 (1993) 600–608.

[23] T.E. Freyaldenhoven. S.F. Ali. R.W. Hart. MPTP- and MPP(+)-induced effects on body temperature exhibit age- and strain-dependence in mice. Brain Res. 688 (1995) 161–170.

[24] T.E. Freyaldenhoven. J.L. Cadet. S.F. Ali. The dopamine-depleting effects of 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine in GD-1 mice are gender-dependent. Brain Res. 735 (1996) 232–238.

[25] R.R. Gainetdinov. F. Fumagalli. S.R. Jones. M.G. Caron. Dopamine transporter is required for in vivo MPTP neurotoxicity: evidence from mice lacking the transporter. J. Neurochem. 69 (1997) 1322–1325.

[26] T. Gasser. B. Müller-Myhsok. Z.K. Wszolek. R. Oehlmann. D.B. Calne. V. Bonifati. B. Bereznai. E. Fabrizio. P. Vieregge. R.D. Horstmann. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat. Genet. 18 (1998) 262–265.

[27] D.C. German. E.L. Nelson. C.-L. Liang. S.G. Speciale. C.M. Sinton. P.K. Sonsalla. The neurotoxin MPTP causes degeneration of specific nucleus A8. A9 and A10 dopaminergic neurons in the mouse. Neurodegeneration 5 (1996) 299–312.

[28] W.R.G. Gibb. A.J. Lee. The significance of the Lewy body in the diagnosis of idiopathic Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 54 (1989) 388–396.

[29] A. Giovanni. P.K. Sonsalla. R.E. Heikkila. Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine. Part 2: Central administration of 1-methyl-4-phenylpyridinium. J. Pharmacol. Exp. Ther. 270 (1994) 1008–1014.

[30] V. Glover. C. Gibb. M. Sandler. The role of MAO in MPTP toxicity —a review. J. Neural Trans. 20 (1986) 65–76.

[31] L.I. Golbe. J.W. Langston. The etiology of Parkinson's disease: new directions for research. in: J. Jankovic. E. Tolosa (Eds.). Parkinson's Disease and Movement Disorders. Williams and Wilkins. Baltimore. 1993. pp. 93–101.

[32] A.S. Hazell. Y. Itzhak. H. Liu. M.D. Norenberg. 1-Methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP) decreases glutamate uptake in cultured astrocytes. J. Neurochem. 68 (1997) 2216–2219.

[33] R.E. Heikkila. Differential neurotoxicity of 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP) in Swiss–Webster mice from different sources. Eur. J. Pharmacol. 117 (1985) 131–133.

[34] M.M. Hoehn. M.D. Yahr. Parkinsonism: onset. progression and mortality. Neurology 17 (1967) 427–442.

[35] T. Hokfelt. R. Martensson. A. Bjorkland. S. Kleinau. M. Goldstein. Distributional maps of tyrosine–hydroxylase–immunoreactive neurons in the rat brain. in: A. Bjorkland. T. Hokfelt (Eds.). Handbook of Chemical Neuroanatomy. Vol. 2. Elsevier. Amsterdam. 1984. pp. 277–379.

[36] S.M. Humphrey. J.B. Gavin. R.N. Seelye. V.J. Webster. Interrelationships of loss of vascular competence. ATP depletion. and de-

creased tissue compressibility in developing myocardial infarcts. Biochem. Med. 24 (1980) 6–15.
[37] K. Ishiwata, T. Ido, K. Yanai, K. Kawashima, Y. Miura, M. Monma, S. Watanuki, T. Takahashi, R. Iwata, Biodistribution of a positron-emitting suicide inactivator of monoamine oxidase, carbon-11 pargyline, in mice and a rabbit, J. Nucl. Med. 26 (1985) 630–636.
[38] J.N. Johannessen, C.C. Chiueh, J.P. Bacon, N.A. Garrick, R.S. Burns, V.K. Weise, I.J. Kopin, J.E. Parisi, S.P. Markey, Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the dog: effect of pargyline pretreatment, J. Neurochem. 53 (1989) 582–589.
[39] M. Kilbourn, K. Frey, Striatal concentrations of vesicular monoamine transporters are identical in MPTP-sensitive (C57BL/6) and -insensitive (CD-1) mouse strains, Eur. J. Pharmacol. 307 (1996) 227–232.
[40] T. Kitada, S. Asakawa, N. Hattori, H. Matsumine, Y. Yamamura, S. Minoshima, M. Yokochi, Y. Mizuno, N. Shimizu, Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism, Nature 392 (1998) 605–608.
[41] B.W. Konigsmark, Methods for counting of neurons, in: W.J.H. Nauta, E.B.E. Ebbeson (Eds.), Contemporary Research Methods in Neuroanatomy, Springer, New York, 1970, pp. 315–380.
[42] A. Kupsch, M. Gerlach, S.C. Pupeter, J. Sautter, A. Dirr, G. Arnold, W. Opitz, H. Przuntek, P. Riederer, W.H. Oertel, Pretreatment with nimodipine prevents MPTP-induced neurotoxicity at the nigral, but not at the striatal level in mice, Neuroreport 6 (1995) 621–625.
[43] M.C. Kurth, J.H. Kurth, Variant cytochrome *P*450 CYP2D6 allelic frequencies in Parkinson's disease, Am. J. Med. Genet. 48 (1993) 166–168.
[44] J.W. Langston, The case of the tainted heroin, The Sciences 25 (1985) 34–40.
[45] J.W. Langston, P. Ballard, J.W. Tetrud, I. Irwin, Chromic parkinsonism in humans due to a product of merperidine-analog synthesis, Science 219 (1983) 979–980.
[46] J.W. Langston, E.B. Langston, I. Irwin, MPTP-induced parkinsonism in human and non-human primates-clinical and experimental aspects, Acta Neurol. Scand. 100 (1984) 49–54.
[47] M.J. Marks, J.A. Stitzel, A.C. Collins, Genetic influences on nicotine responses, Pharmacol. Biochem. Behav. 33 (1989) 667–678.
[48] C.D. Marsden, C.W. Olanow, The causes of Parkinson's disease are being unraveled and rational neuroprotective therapy is close to reality, Ann. Neurol. 44 (1998) S189–196, Review; 25 refs.
[49] T. May, M. Pawlik, H. Rommelspacher, [3H]harman binding experiments: II. Regional and subcellular distribution of specific [3H]harman binding and monoamine oxidase subtypes A and B activity in marmoset and rat, J. Neurochem. 56 (1991) 500–508.
[50] R. Mayeux, J. Denaro, N. Hemenegildo, K. Marder, M.-X. Tang, L.J. Cote, Y. Stern, A population-based investigation of Parkinson's disease with an without dementia. Relationship to age and gender, Arch. Neurol. 49 (1992) 492–497.
[51] G.A. Milliken, Analysis of messy data, Design of Experiments, Vol. 1, Chapman & Hall, New York, 1992.
[52] E.L. Nelson, C.-L. Liang, C.M. Sinton, D.C. German, Midbrain dopaminergic neurons in the mouse: computer-assisted mapping, J. Comp. Neurol. 369 (1996) 361–371.
[53] M. Neystat, C.M. Troy, T. Lynch, T. Vu, S. Przedborski, Differential display in PC12 cells exposed to MPP+, Soc. Neurosci. Abstr. 23 (1997) 1654.
[54] R.L. Nussbaum, M.H. Polymeropoulos, Genetics of Parkinson's disease, Hum. Mol. Genet. 6 (1997) 1687–1692.
[55] B. Pakkenberg, A. Moller, H.J. Gunderson, A. Mouritzen Dam, H. Pakkenberg, The absolute number of nerve cells of the substantia nigra in normal subjects and patients with Parkinson's disease estimated with an unbiased steriological method, J. Neurol. Neurosurg. Psychiatry 54 (1991) 30–33.
[56] J. Parkinson, An Essay on Shaking Palsy, Sherwood, Neeley and Jones, London, 1817.
[57] A.H. Paterson, Molecular dissection of quantitative traits: progress and prospects, Genome Res. 5 (1995) 321–333.
[58] C. Pifl, B. Giros, M.G. Caron, Dopamine transporter expression confers cytotoxicity to low doses of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium, J. Neurosci. 13 (1993) 4246–4253.
[59] R. Plomin, G.E. McClearn, G. Gora-Maslak, R.J.M. Neiderhise, Use of recombinant inbred strains to detect quantitative trait loci associated with behavior, Behav. Genet. 21 (1991) 99–116.
[60] M.H. Polymeropoulos, C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, R. Boyer, E.S. Stenroos, S. Chandrasekharappa, A. Athanassiadou, T. Papapetropoulos, W.G. Johnson, A.M. Lazzarini, R.C. Duvoisin, G.' Di Iorio, L.I. Golbe, R.L. Nussbaum, Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, Science 276 (1997) 2045–2047.
[61] D.C. Poskanzer, R.S. Schwab, Cohort analysis of Parkinson's syndrome:evidence for a single etiology related to subclinical infection about 1920, J. Chron. Dis. 16 (1963) 961–973.
[62] S. Przedborski, V. Kostic, V. Jackson-Lewis, A.B. Naini, S. Simonetti, S. Fahn, E. Carlson, C.J. Epstein, J.L. Cadet, Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity, J. Neurosci. 12 (1992) 1658–1667.
[63] A.H. Rajput, Epidemiology of Parkinson's disease, Can. J. Neurol. Sci. 11 (1984) 156–159.
[64] K.A. Reimer, R.B. Jennings, A.H. Tatum, Pathobiology of acute myocardial ischemia: metabolic, functional and ultrastructural studies, Am. J. Cardiol. 52 (1983) 72A–81A.
[65] N.J. Riachi, S.I. Harik, Strain differences in systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in mice correlate best with monoamine oxidase activity at the blood–brain barrier, Life Sci. 42 (1988) 2359–2363.
[66] M.S. Sandy, M. Armstrong, C.M. Tanner, A.K. Daly, D.A. Di Monte, J.W. Langston, J.R. Idle, CYP2D6 allelic frequencies in young-onset Parkinson's disease, Neurology 47 (1996) 225–230.
[67] SAS Institute, SAS/STAT User's Guide, Version 6, SAS Institute, NC, 1989.
[68] J. Saura, E. Nadal, B. van den Berg, M. Vila, J.A. Bombi, N. Mahy, Localization of monoamine oxidases in human peripheral tissues, Life Sci. 59 (1996) 1341–1349.
[69] A.H. Schapira, Nuclear and mitochondrial genetics in Parkinson's disease, J. Med. Genet. 32 (1995) 411–414.
[70] J.S. Schneider, C.H. Markham, Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the cat, Tyrosine hydroxylase immunohistochemistry, Brain Res. 373 (1986) 258–267.
[71] F. Seitelberger, General neuropathology of degenerative processes of the nervous system, Neurosci. Res. 2 (1969) 253–299, Review; 30 refs.
[72] Y. Singh, E. Swanson, E. Sokoloski, R.K. Kutty, G. Krishna, MPTP and MPTP analogs induced cell death in cultured rat hepatocytes involving the formation of pyridinium metabolites, Toxicol. Appl. Pharmacol. 96 (1988) 347–359.
[73] E. Sofic, W. Paulus, K. Jellinger, P. Riederer, M. Youdim, Selective increase in iron in substantia nigra zona compacta of parkinsonian brains, J. Neurochem. 56 (1991) 978–982.
[74] P.K. Sonsalla, R.E. Heikkila, Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice, Prog. Neuropsychopharmacol. Biol. Psychiatry 12 (1988) 345–354.
[75] M.G. Spillantini, M.L. Schmidt, V.M. Lee, J.Q. Trojanowski, R. Jakes, M. Goedert, Alpha-synuclein in Lewy bodies, Nature 388 (1997) 839–840.
[76] A. Stenstrom, E. Sundstrom, C.J. Fowler, Comparison of intra- and extrasynaptosomal monoamine oxidase-A and -B activities in the striatum and frontal cortex of two mice strains with different sensitivities to the neurotoxic actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Pharmacol. Toxicol. 64 (1989) 276–281.
[77] E. Sundstrom, I. Stromberg, T. Tsutsumi, L. Olson, G. Jonsson, Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyri-

dine (MPTP) on central catecholamine neurons in C57Bl/6 mice. Comparison with three other strains of mice. Brain Res. 405 (1987) 26–38.

[78] R.H. Swerdlow, J.K. Parks, S.W. Miller, J.B. Tuttle, P.A. Trimmer, J.P. Sheehan, J.P.J. Bennett, R.E. Davis, W.D.J. Parker, Origin and functional consequences of the complex I defect in Parkinson's disease, Ann. Neurol. 40 (1996) 663–671.

[79] Q. Wei, M. Yeung, O.P. Jurma, J.K. Andersen, Genetic elevation of monoamine oxidase levels in dopaminergic PC12 cells results in increased free radical damage and sensitivity to MPTP, J. Neurosci. Res. 46 (1996) 666–673.

[80] R.W. Williams, R.C. Strom, D. Goldowitz, Natural variation in neuron number in mice is linked to a major quantitative trait locus on Chr 11, J. Neurosci. 18 (1998) 138–146.

[81] R.W. Williams, R.C. Strom, D.S. Rice, D. Goldowitz, Genetic and environmental control of variation in retinal ganglion cell number in mice, J. Neurosci. 16 (1996) 7193–7205.

[82] L. Wolman, S. Roy, The substantia nigra in Parkinsonism, J. Clin. Pathol. 22 (1969) 507–508.

[83] M.B. Youdim, D. Ben-Shachar, P. Riederer, Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced neurodegeneration?, Acta Neurol. Scand. 126 (1989) 47–54.

[84] L.Y. Zang, H.P. Misra, Generation of reactive oxygen species during the monoamine oxidase catalyzed oxidation of the neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetra hydropyridine, J. Biol. Chem. 268 (1993) 16504–16512.